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Potent Preventive Action of α -Carotene against Carcinogenesis: Spontaneous Liver Carcinogenesis and Promoting Stage of Lung and Skin Carcinogenesis in Mice Are Suppressed More Effectively by α -Carotene Than by β -Carotene¹

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ABSTRACT

Although β -carotene has been considered to be a key cancer preventive agent in green and yellow vegetables, other types of carotenoids, such as α -carotene, may also contribute to anticarcinogenic action, since these carotenoids usually coexist with β -carotene and are detectable in human blood and tissues. In this study, we compared the inhibitory effect of natural α -carotene, obtained from palm oil, with that of β -carotene on spontaneous liver carcinogenesis in C3H/He male mice. The mean number of hepatomas per mouse was significantly decreased by α -carotene supplementation (*per os* administration in drinking water at a concentration of 0.05%, *ad libitum*) as compared with that in the control group ($P < 0.001$, Student's *t* test). On the other hand, β -carotene, at the same dose as α -carotene, did not show any such significant difference from the control group. Furthermore, we also compared the antitumor-promoting activity of α -carotene with that of β -carotene against two-stage mouse lung carcinogenesis (initiator, 4-nitroquinoline 1-oxide; promoter, glycerol). α -Carotene, but not β -carotene, reduced the number of lung tumors per mouse to about 30% of that in the control group ($P < 0.001$, Student's *t* test). The higher potency of the antitumor-promoting action of α -carotene compared to β -carotene was confirmed in other experimental systems; e.g., α -carotene was also found to have a stronger effect than β -carotene in suppressing the promoting activity of 12-*O*-tetradecanoylphorbol-13-acetate on skin carcinogenesis in 7,12-dimethylbenz[*a*]anthracene-initiated mice.

These results suggest that not only β -carotene, but also other types of carotenoids, such as α -carotene, may play an important role in cancer prevention.

INTRODUCTION

Epidemiological investigations have shown that cancer risk is inversely related to the consumption of green and yellow vegetables (1-7). Since β -carotene presents in abundance in green and yellow vegetables and has the highest provitamin A activity, β -carotene has been proposed as a key cancer preventive agent. In fact, experiments have confirmed that β -carotene prevented or delayed carcinogenesis induced by chemicals (8-10) and viruses (11). However, it should also be noted that β -carotene is often associated with other types of natural carotenoids, such as α -carotene, lycopene, etc. in daily foodstuffs (Table 1) (12). Furthermore, not only β -carotene, but also these carotenoids, are detectable in human serum (13) and in a wide variety of tissues (14). Therefore, it is of interest to investigate the biological activity of these various kinds of carotenoids more extensively.

In recent studies, we found that α -carotene inhibited the proliferation of human malignant tumor cells more effectively than β -carotene (15, 16). In the present study, we further compared the activity of α -carotene with that of β -carotene against carcinogenesis.

MATERIALS AND METHODS

Chemicals. Palm oil carotene, extracted from palm oil by a previously reported method (17), with slight modifications, was provided by Lion Oleo-Chemical Co. (Tokyo, Japan). The component percentages of palm oil carotene were almost the same as those of carrot carotene: 30% α -carotene; 60% β -carotene; and 10% others (γ -carotene, lycopene, etc.) (Fig. 1). α -Carotene was purified from palm oil carotene by high-performance liquid chromatography with a lime-packed column. No impurity was seen in α -carotene on high-performance liquid chromatography. β -Carotene was purchased from Sigma Chemical Co. (St. Louis, MO). α -Carotene, β -carotene, and palm oil carotene were prepared as emulsions with 0.5% sucrose ester P-1570 (Mitsubishi-Kasei Food Co., Tokyo, Japan), 1.0% Sansoft 8000 (Taiyo Co., Tokyo, Japan), 0.2% L-ascorbyl stearate, and 4% peanut oil. 4NQO³ and glycerol were purchased from Nacalai Tesque Co. (Kyoto, Japan). DMBA was purchased from Wako Pure Chemical Industries (Osaka, Japan). TPA was obtained from Pharmacia LKB Biotechnology Japan (Tokyo, Japan). D,L-[1-¹⁴C]Ornithine hydrochloride was obtained from New England Nuclear (Boston, MA).

In Vivo Mouse Spontaneous Liver Carcinogenesis Experiment. Male C3H/He mice, which have a high incidence of spontaneous liver tumor development, were used. Eight-week-old mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). α -Carotene, β -carotene, or palm oil carotene (at concentrations of 0.005% or 0.05%) or vehicle for emulsion sample was mixed in drinking water and given *ad libitum* for 40 weeks. In the preparation of the drinking water, each carotene was prepared as an emulsion to make up final concentrations of 0.005% or 0.05% of the water. Drinking water was prepared every 3 days. Carotene was stable for at least 3 days. Each experimental group consisted of 17 mice.

Mice were killed at week 40 by cervical dislocation, following which they were autopsied, and the number of liver tumor nodules was measured.

In Vivo Two-Stage Mouse Lung Carcinogenesis Experiment. This experiment was performed as reported by Inayama (18). The animals used were 6-week-old male mice, specific pathogen-free ddY strain (purchased from Shizuoka Laboratory Animal Center). 4NQO was dissolved in a mixture of olive oil and cholesterol (20:1), and 10 mg/kg body weight (about 0.3 mg/mouse) was given by single s.c. injection on the first experimental day. A 10% solution of glycerol in water was given as drinking water *ad libitum* from the beginning of experimental week 5 continuously for 25 weeks. α - or β -Carotene (at a concentration of 0.05%) or emulsion vehicle was mixed into the drinking water. Each experimental group consisted of 16 mice.

Mice were killed at week 30 by cervical dislocation. At autopsy, the lungs were fixed via intratracheal instillation of 10% formaldehyde.

³ The abbreviations used are: 4NQO, 4-nitroquinoline 1-oxide; DMBA, 7,12-dimethylbenz[*a*]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ODC, ornithine decarboxylase.

Received 11/25/91; accepted 9/23/92.

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¹ Supported in part by grants from the Ministry of Education, Science, and Culture; the Smoking Research Foundation; and the Plant Science Research Foundation, Faculty of Agriculture, Kyoto University, Japan.

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Table 1 Carotenoid content of selected foods^a

All amounts are measured in mg/lb. Amounts of carotenoids less than 20 mg/lb are not reported.

Food	Total carotenoids	β -Carotene	Other
Plant ^b			
Broccoli	20	7	Lutein: 4
Carrot	85	51	α -Carotene: 27
Kale	130	35	Lutein: 9
Lettuce	44	10	
(Romaine)			
Mustard greens	93	30	Lutein: 45
Potato, sweet	62	55	
Pumpkin	130	72	α -Carotene: 55
Scallion	22	6	Lutein: 10
Spinach	123	31	Lutein: 59
Squash	28	10	Lutein: 7
Tomato	45	5	Lycopene: 40
Watermelon	22		Lycopene: 22
Animal			
Liver	160	14	Vitamin A: 130

^a See Ref. 12.

^b Vegetables are listed alphabetically.

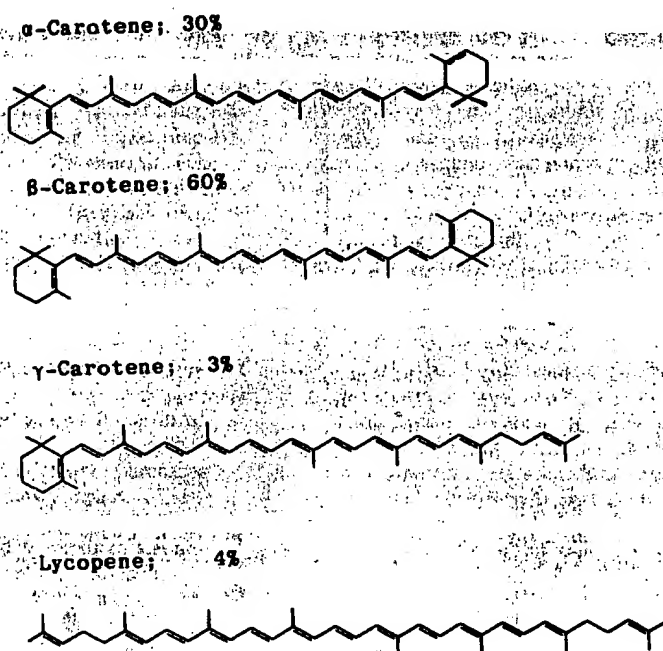


Fig. 1. Constituents of palm oil carotene.

After separation of each pulmonary lobe, the number of induced tumors was counted under a microscope.

In Vivo Two-Stage Mouse Skin Carcinogenesis Experiment. Seven-week-old ICR mice (purchased from Shizuoka Laboratory Animal Center) had their backs shaved with an electric clipper. After 2 days, initiation was accomplished by a single application of DMBA (100 μ g) on the shaved area. TPA, at the dose of 1 μ g (1.62 nmol)/painting was applied twice a week starting 1 week after initiation. α - or β -Carotene (200 or 400 nmol), mixed in 200 μ l of acetone, was applied simultaneously with each application of promoter. Controls were treated with vehicle in acetone. The experiment was continued for 20 weeks.

Each experimental group consisted of 16 mice. The number of tumors was determined once a week.

Assay of Ornithine Decarboxylase Activity. The backs of 7-week-old ICR mice were shaved with an electric clipper. TPA (17 nmol) was applied with 200 or 400 nmol of α -carotene, β -carotene, palm oil carotene, or vehicle in 200 μ l acetone. After 4 h, mice were killed by cervical dislocation, and the epidermises were separated from individual

mice after brief heat treatment (55°C for 30 s). The epidermal preparations were pooled, homogenized in 50 mM sodium phosphate buffer (pH 7.2) containing 0.1 mM pyridine phosphate and 0.1 mM EDTA, and then centrifuged (30,000 \times g for 30 min). ODC activity in soluble epidermal extract was determined by measuring the release of 14 CO₂ from DL-[1- 14 C]ornithine hydrochloride. Protein content in the soluble epidermal extract was determined by the method of Lowry *et al.* (19).

RESULTS

Effects of α -Carotene, β -Carotene, and Palm Oil Carotene on Spontaneous Liver Carcinogenesis. Recently, we found that administration p.o. of palm oil carotene (0.005%), mixed in drinking water, resulted in a decrease of the mean number of tumors per mouse, as compared with the control group ($P < 0.01$, Student's *t* test), in spontaneous liver carcinogenesis in C3H/He male mice.⁴ In the present study, we further examined the inhibitory effects of α -carotene, β -carotene, and palm oil carotene (at concentrations of 0.005% and 0.05% in drinking water) on this spontaneous liver carcinogenesis.

There were no significant differences in final body weight or in mean amount of drinking water consumed among the groups (Table 2). Mean α -, β -, and palm oil carotene intake, calibrated from the mean amount of drinking water consumed, is summarized in Table 2.

As shown in Table 3, the mean number of hepatomas was significantly decreased by oral administration of 0.05% α -carotene as compared with that in the control group; the control group developed 6.31 ± 0.62 tumors/mouse (mean \pm SE), whereas the 0.05% α -carotene-treated group had 3.00 ± 0.36 tumors/mouse ($P < 0.001$, Student's *t* test). On the other hand, the 0.05% β -carotene-treated group did not show a significant difference from the control group, although a tendency toward a decrease in the mean number of hepatomas was observed. At a concentration of 0.005%, neither α - nor β -carotene had any significant anticarcinogenic effects.

From these results, it is apparent that the inhibitory effect of α -carotene on the development of spontaneous mouse liver carcinogenesis is stronger than that of β -carotene. It is noteworthy that the palm oil carotene-treated groups also showed significant differences from the control group in the incidence of tumors; i.e., the mean number of tumors per mouse in the 0.005% and 0.05% palm oil carotene groups was 3.60 ± 0.40 ($P < 0.01$, Student's *t* test) and 2.06 ± 0.37 ($P < 0.001$, Student's *t* test), respectively. Treatment with palm oil carotene appeared to have a greater effect than treatment with α -carotene. There were no differences in the sizes and histological findings of the tumors among the groups. There is an ill circumscribed tumor in the noncirrhotic liver tissue. The tumor cells are arranged in thin trabecular structures surrounded by sinusoidal structures. The nuclei of the tumor cells are slightly enlarged, and mitoses are seen sporadically, but pleomorphism is not so profound and the tumor is identified as well-differentiated hepatocellular carcinoma.

Effects of α -Carotene and β -Carotene on Two-Stage Mouse Lung Carcinogenesis. We also examined the effects of α - and β -carotene on the promotion of lung tumor formation in 4NQO-initiated mice. As shown in Table 4, oral administration of α -carotene resulted in a decrease of the mean number of tumors per mouse, to about 30% of the number in the control group ($P < 0.001$, Student's *t* test). The β -carotene-treated

⁴ H. Nishino *et al.*, unpublished observations.

Table 2 Body weight and carotene intake during spontaneous mouse liver carcinogenesis

Eight-week-old male C3H/He mice were used in this experiment. α -, β -, and palm oil carotene (at the concentration of 0.005% or 0.05%) or vehicle emulsion as control was mixed in drinking water and given p.o. for 40 weeks.

Group	n	Body Weight (g) ^a		Mean amount of drinking water consumed (ml/mouse/day) ^a	Mean α -, β -, or palm oil carotene intake (mg/mouse/day)
		Week 0	Week 40		
Control	17	26.4 \pm 0.21	38.5 \pm 0.63	5.00 \pm 0.06	
+0.005% α -carotene	17	26.5 \pm 0.18	38.9 \pm 1.00	4.98 \pm 0.06	0.25
+0.05% α -carotene	17	26.3 \pm 0.24	40.0 \pm 0.71	4.82 \pm 0.08	2.41
+0.005% β -carotene	17	26.3 \pm 0.25	39.7 \pm 0.75	4.86 \pm 0.06	0.24
+0.05% β -carotene	17	26.7 \pm 0.36	37.4 \pm 0.65	4.94 \pm 0.06	2.47
+0.005% Palm oil carotene	17	26.2 \pm 0.14	39.6 \pm 0.81	4.91 \pm 0.07	0.25
+0.05% Palm oil carotene	17	26.4 \pm 0.14	39.0 \pm 0.91	5.14 \pm 0.06	2.57

^a Mean \pm SE.

Table 3 Effects of α -, β -, and palm oil carotene on spontaneous mouse liver carcinogenesis

At week 40, mice were killed by cervical dislocation and autopsied. The number of liver tumor nodules was then measured.

Group	n	Number of tumor-bearing mice (%)	Mean number of tumors/mouse ^a
Control	16	16 (100)	6.31 \pm 0.62
+0.005% α -carotene	15	15 (100)	5.07 \pm 0.49
+0.05% α -carotene	17	16 (94)	3.00 \pm 0.36 ^b
+0.005% β -carotene	16	16 (100)	7.38 \pm 0.83
+0.05% β -carotene	17	17 (100)	4.71 \pm 0.39
+0.005% Palm oil carotene	15	15 (100)	3.60 \pm 0.40 ^c
+0.05% Palm oil carotene	16	13 (81)	2.06 \pm 0.37 ^b

^a Mean \pm SE.

^b $P < 0.001$, as compared with control group.

^c $P < 0.01$, as compared with control group.

group, on the other hand, did not show such significant difference from the control group. α -Carotene, but not β -carotene, also showed a tendency toward decreasing the percentage of tumor-bearing mice, although the difference was not statistically significant. There were no differences in the sizes of the tumors among the groups. The antitumorpromoting activity did not appear to be the result of general carotenoid toxicity, since no significant difference in growth was noted among the groups, i.e., the mean final body weights in the control, α -carotene, and β -carotene groups were 50.6 \pm 6.00, 49.8 \pm 4.38, and 50.6 \pm 6.22 g (mean \pm SE), respectively. There was no significant difference in water intake among the groups; the mean amount of drinking water consumed was 5.71 \pm 0.18, 6.53 \pm 1.05, and 6.53 \pm 0.28 ml/mouse/day (mean \pm SE), respectively. The mean α - and β -carotene intake, calculated from the mean amount of drinking water consumed, was found to be at the same level in the two carotenoid-treated groups. Histologically, most tumor nodules were considered to be so-called type II adenoma.

The repeated experiment showed almost the same results. From all of these results taken together, it is apparent that

α -carotene has more effective antitumorpromoting activity than β -carotene in lung carcinogenesis.

Effects of α -Carotene and β -Carotene on Two-Stage Mouse Skin Carcinogenesis. The greater potency of α -carotene than β -carotene in the suppression of tumor promotion was confirmed by other two-stage carcinogenesis experiments *in vivo*.

Fig. 2 shows the time course of skin tumor (papilloma) formation in the groups treated with DMBA plus TPA, with or without 200 or 400 nmol of α -carotene or β -carotene. The mean final body weights for the control and the 200 and 400 nmol α - and β -carotene groups were 45.3 \pm 0.95, 44.0 \pm 1.69, 45.4 \pm 1.56, 46.96 \pm 1.98, and 45.21 \pm 1.70 g (mean \pm SE), respectively. There was no significant difference among the groups.

In the control and β -carotene-treated groups, the first tumor appeared within 9 weeks of promotion, and in the α -carotene-treated groups, the first tumor appeared at week 13. The development rate of tumors was higher in the control group than in the carotenoid-treated groups. The comparison of control and carotenoid-treated groups at week 20 can be summarized as follows.

The percentage of tumor-bearing mice in the control group was 69%, whereas the percentages in the groups treated with 200 and 400 nmol of α -carotene or β -carotene were 25%, 13%, 31%, and 25%, respectively (Fig. 2A). Though both α - and β -carotene inhibited the promoting activity of TPA, α -carotene was more inhibitory than β -carotene.

α -Carotene also decreased the average number of tumors per mouse. The control group developed 3.8 tumors/mouse, whereas the 200 and 400 nmol α -carotene groups had 0.3 ($P < 0.01$, Student's *t* test) and 0.1 ($P < 0.01$) tumors/mouse, respectively (Fig. 2B). β -Carotene treatment also decreased the average number of tumors per mouse, but the difference from the control group was not significant. The size of tumors was smaller in the groups treated with carotenoids than that in the control group (Table 5).

Almost the same result was obtained in the repeated experiment. These results confirm that α -carotene has a stronger antitumor-promoting effect than β -carotene.

Table 4 Effects of α - and β -carotene on the promotion of lung tumor formation by glycerol in 4NQO-initiated mice

4NQO, dissolved in a mixture of olive oil and cholesterol (20:1), was given by a single s.c. injection on the first experimental day (10 mg/kg body weight of 4NQO). Glycerol was dissolved in water and the 10% solution was given as drinking water *ad libitum* from experimental week 5 to week 30 continuously. α - and β -carotene (at the concentration of 0.05%) or vehicle as a control was mixed as an emulsion into drinking water. Each experimental group consisted of 16 mice.

Group	Mean amount of drinking water consumed (ml/mouse/day) ^a	Mean α - or β -carotene intake (mg/mouse/day)	Percentage of tumor-bearing mice	Mean number of tumors/mouse ^a
Control	5.71 \pm 1.03		94	4.06 \pm 0.18
+ α -Carotene	6.53 \pm 1.05	3.27	73	1.33 \pm 0.08 ^b
+ β -Carotene	6.53 \pm 1.17	3.27	93	4.93 \pm 0.28

^a Mean \pm SE.

^b Significant difference from control group ($P < 0.001$).

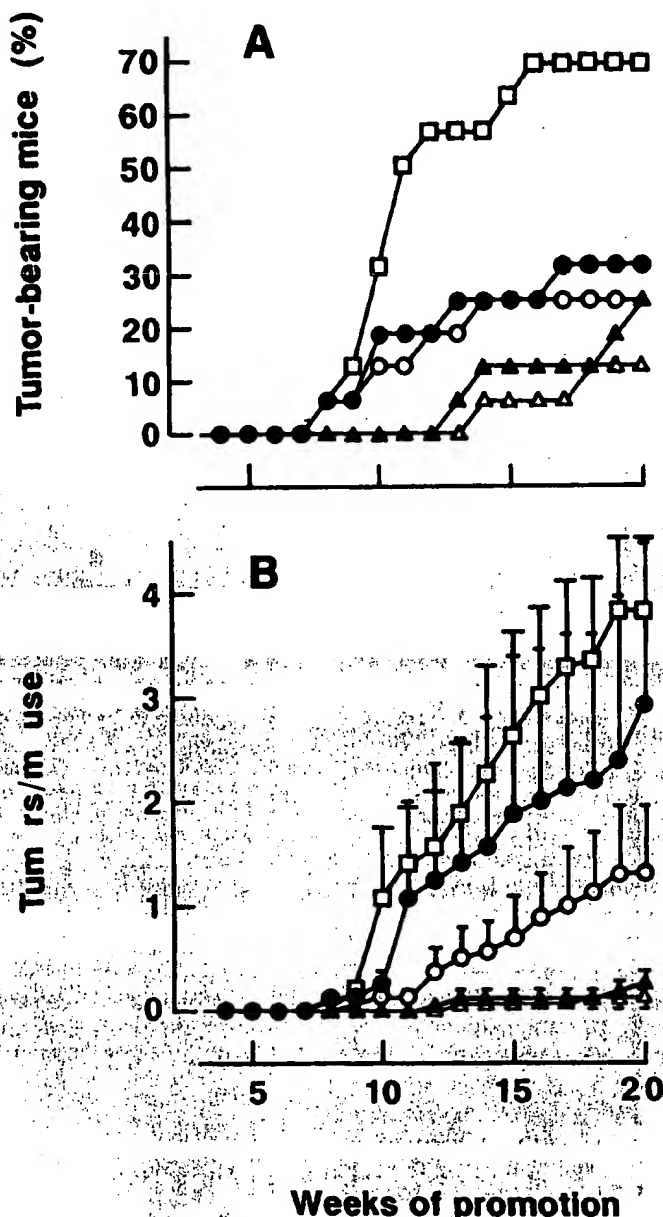


Fig. 2. Effects of α - and β -carotene on the promotion of skin tumor formation by TPA in DMBA-initiated mice. From 1 week after initiation by 100 μ g of DMBA, 1.0 μ g of TPA was applied twice a week. α - or β -Carotene (200 or 400 nmol) was applied with each TPA application. A, percentage of tumor-bearing mice; B, tumor incidence \pm SE. \square , group treated with DMBA plus TPA and vehicle as control; Δ , \triangle , groups treated with DMBA plus TPA and 200 or 400 nmol α -carotene, respectively; \bullet , \circ , groups treated with DMBA plus TPA and 200 or 400 nmol β -carotene, respectively. Each experimental group consisted of 16 mice.

Effects of α -Carotene and β -Carotene on TPA-induced ODC Activity in Mice. As shown in Table 6, the application of α -carotene, β -carotene, or palm oil carotene suppressed the induction of ODC activity by a tumor promoter in a dose-dependent manner.

The inhibitory effect of α -carotene was greater than that of β -carotene. Furthermore, it is worthy of note that treatment with palm oil carotene had the greatest effect.

DISCUSSION

Carotenes and retinols in excess of the body's immediate needs are stored mainly in the liver (20). The total amount of

carotenoids present in the human body has been estimated to be about 140 mg, and approximately 10% of this total is found in the liver (21, 22). Therefore, it is of interest to investigate the effect of various kinds of carotenoids on liver carcinogenesis. The present findings clearly demonstrate that α -carotene has a potent inhibitory effect on spontaneous liver carcinogenesis. It is noteworthy that α - but not β -carotene showed potent inhibitory activity against liver carcinogenesis in this experiment.

In recent years, the incidence of lung cancer has gradually increased. Since the lung has been suggested as a possible target organ for the suppression of carcinogenesis by carotenoids, we compared the antitumor-promoting activity of α -carotene with that of β -carotene in two-stage mouse lung carcinogenesis (initiator, 4NQO; promoter, glycerol). α -Carotene was more effective than β -carotene in inhibiting the tumor-promoting action of glycerol in this experimental system.

α -Carotene also had a greater inhibitory effect than β -carotene on TPA-induced skin tumor promotion. The correlation between the ability of various compounds to inhibit TPA-induced ODC activity and their ability to inhibit the formation of skin papillomas has been reported previously (23). In our experiment, the inhibitory potency of α - and β -carotene against TPA-induced ODC activity reflected well their protective effects against skin tumor formation. This result indicates that the mechanism underlying the prevention of skin carcinogenesis by α -carotene possibly involves its ability to inhibit TPA-induced ODC activity. In the present study, carotenes were administered simultaneously with TPA. Although a direct chemical reaction between carotenes and TPA is very unlikely, it would be worth investigating such a possibility by studying whether carotenes are also effective, regardless of whether they are applied before or after the treatment with TPA.

The protective effects of β -carotene against tumor formation at several sites in rats, mice, and hamsters have been reported (24–28). In those studies, it was suggested that the protective

Table 5. Effect of α -carotene and β -carotene on the promotion of skin papillomas by TPA in DMBA-initiated mice.

Experimental conditions were as described in Fig. 2. At week 20 of promotion, the diameter and number of tumors were measured.

Condition	Dose (nmol)	Average no. of tumors/mouse					Total
		Size of tumors (mm)					
		1-2	2-3	3-4	4-5	5+	
Control		2.20	1.07	0.20	0.13	0.13	3.73
+ α -Carotene	200	0.20	0.07				0.27
	400	0.13					0.13
+ β -Carotene	200	1.81	1.03	0.13			2.94
	400	1.06	0.25				1.31

Table 6. Effects of α -, β -, and palm oil carotene on TPA-induced ODC activity in mice.

Mice were treated with 17 nmol of TPA and 200 or 400 nmol of α -, β -, and palm oil carotene in 200 μ l acetone. Control was treated with TPA and vehicle in acetone. Mice were killed 4 h after TPA treatment to determine ODC activity. Each value represents the mean \pm SE of enzyme activity determinations in 3 groups of mice.

Treatment	Dose (nmol)	ODC activity (nmol CO ₂ /30 min/mg protein)	Inhibition (%)
Control		2.31 \pm 0.19	
+ α -Carotene	200	1.98 \pm 0.38	14
	400	1.00 \pm 0.17 ^a	57
+ β -Carotene	200	2.10 \pm 0.34	9
	400	1.48 \pm 0.33	36
+Palm oil carotene	200	0.80 \pm 0.21 ^a	65
	400	0.21 \pm 0.06 ^b	91

^a $P < 0.01$, as compared with control group.

^b $P < 0.001$, as compared with control group.

effects of β -carotene reflected its provitamin A activity. However, the provitamin A activity of α -carotene is about one-half that of β -carotene. Therefore, our results indicate that the chemopreventive activity of α -carotene did not involve its conversion into vitamin A. The mechanism underlying the antitumor effects of α - and β -carotene remains to be elucidated.

Palm oil carotene, which consists of 30% α -carotene, 60% β -carotene, and 10% others (γ -carotene, lycopene, etc.), remarkably suppressed spontaneous liver carcinogenesis in C3H/He male mice, more effectively than α - or β -carotene. Palm oil carotene also has greater inhibitory action on TPA-induced ODC activity than α - or β -carotene. These results suggest that not only α - and β -carotene, but also other kinds of natural carotenoids in palm oil carotene, such as γ -carotene and lycopene, have chemopreventive activity. In fact, our preliminary data showed that a crude mixture sample of γ -carotene and lycopene, prepared from palm oil carotene, had a greater inhibitory action on ODC activity than palm oil carotene. Mascio *et al.* (29) showed the slightly higher singlet oxygen quenching ability of lycopene and γ -carotene compared to β -carotene. Kaplan *et al.* (30) reported that lycopene levels in humans, not only in serum but also in most tissues (14) which they studied (liver, adrenals, testes, etc.), were higher than β -carotene levels. They also suggested that the uneven but wide tissue distribution of most dietary carotenoids could indicate an active biological role for these compounds (14). Marchand *et al.* (31) reported that all vegetables, dark green vegetables, cruciferous vegetables, and tomatoes showed a stronger inverse association with lung cancer risk in Hawaii than β -carotene. Ziegler *et al.* (32) suggested that other carotenoids, other constituents of vegetables and fruits, and dietary patterns tightly associated with vegetable and fruit intake need to be explored further as alternatives to the β -carotene hypothesis. The present results further support their hypothesis; *i.e.*, minor constituents of natural carotenoids, such as γ -carotene, lycopene, etc., may also have protective activity against carcinogenesis.

From these results, it would appear that investigation of the biological activity not only of β -carotene, but also of α -carotene and other kinds of carotenoids in daily foods, could be important in understanding cancer chemoprevention.

ACKNOWLEDGMENTS

We wish to thank Dr. N. Kanisawa, Department of Pathology, Yokohama City University School of Medicine, Yokohama, Japan, for his valuable discussion. We are grateful to Lion Oleo-Chemical Co. Ltd., Tokyo, Japan, for providing palm oil carotene samples.

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